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Research Paper
Bone Healing

Cumulative effect of low-level laser therapy and low-intensity pulsed ultrasound on bone repair in rats

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Abstract. Many studies have assessed the effects of either low-level laser therapy (LLLT) or low-intensity pulsed ultrasound (LIPUS) on bone repair; however, an evaluation of the combination of these modalities (LLLT + LIPUS) has not yet been considered. The aim of this study was to demonstrate the effects of LLLT + LIPUS on bone repair. Male Wistar rats (n = 128; four groups of 32) were used; the animals underwent a partial tibial bone osteotomy. One group had the osteotomized limb treated with LLLT, the second group with LIPUS, and the third group with the combined treatment protocols of the LLLT and LIPUS groups; the fourth group received no further treatment (control). Each group was divided into two subgroups for assessment at two different time-points, 14 and 21 days. After the completion of treatment rats were sacrificed and the tibias submitted to a three-point bending test or to histomorphometric analysis. Histological evaluation showed increased bone trabeculae, increased vascularization, and decreased inflammation in the LLLT + LIPUS group. Mechanical evaluation revealed increased biomechanical properties including maximum force, maximum stress, and stiffness, in the LLLT + LIPUS group. Combined LLLT + LIPUS treatment enhanced bone healing both histologically and mechanically, shortening the length of the treatment period, when compared to treatment with LLLT or LIPUS alone.

Keywords: Bone repair; Healing; Biostimulation; Laser; Ultrasound; LLLT; LIPUS; Combined; Cumulative.

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Bone healing is the replacement of affected cells with others of the same kind, resulting in nearly perfect reconstitution of the normal structures[1]. The process presents the recapitulation of certain aspects of skeletal development and growth, involving a complex interplay of cells, growth factors, and extracellular matrix[2]. A variety of interventions aimed at reducing the length of the treatment period and the high socioeconomic costs have been studied, including the use of low-intensity pulsed ultrasound (LIPUS) and low-level laser therapy (LLLT)[3].

Clinically, LIPUS produces significant responses from cells and tissues, including degranulating supporting cells; altering the function of the cellular membrane thereby increasing intracellular calcium levels; stimulating fibroblastic activity thereby increasing protein synthesis; and increasing angiogenesis and vascular permeability[4]. An alternative form of treatment is laser irradiation, which can increase the structural stiffness of the bone callus[3]. Some authors affirm that LLLT can accelerate bone formation by increasing osteoblastic activity[5,6], vascularization[7], organization of collagen fibers[8], and adenosine triphosphate (ATP) levels[9].

Studies comparing the effects of LIPUS and LLLT on bone healing are limited[3,10,11], and the cumulative effect of both LIPUS and LLLT on the different phases of bone healing has not yet been assessed. The aim of this study was to evaluate the histological and mechanical changes in bone tissue when the effects of LIPUS and LLLT are combined.

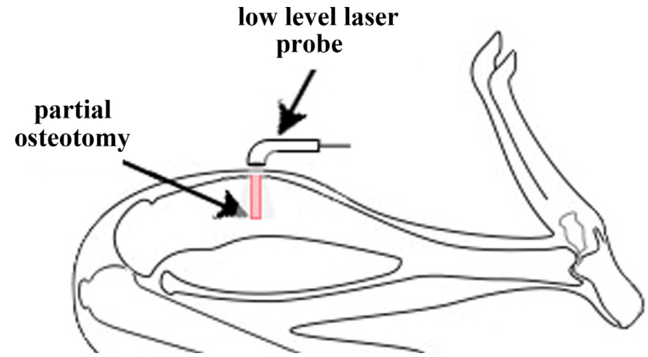

Fig. 1. Application of the low-level laser device.

Methods

Surgery

Healthy male albino Wistar rats ($n = 128$), weighing an average of 300 g, were used in this study. Under general anaesthesia (ketamine), the right tibia of each animal was surgically exposed, and a partial transverse osteotomy was made under saline irrigation distal to the anterior part of the right knee using a 1-mm diameter drill. The skin was then sutured and cleaned. The animals were kept in cages of four each, under appropriate light and temperature conditions, and had access to water and food ad libitum. The 128 rats were randomly allocated to the four study groups (32 in each): LLLT, LIPUS, LLLT + LIPUS, and a control group. Each group was then further divided into two subgroups of 16 animals for assessment at two different time-points–following a treatment period of either 14 or 21 days.

Treatment

Thirty-two rats underwent the osteotomy only and comprised the control group. In the LLLT group, 32 rats underwent the osteotomy and were treated with a low-level laser (Doris Duo CTL 1106MX; Ga–Al–As laser, 820 nm, 1 cm^2 beam area, 0.5 W, 16 J/cm^2; CTL, Warsaw, Poland) every other day, beginning on the day of surgery (Fig. 1). The LIPUS group rats ($n = 32$) were treated with a therapeutic ultrasound device (Intelect 340 Combo; 1 MHz, 1:4 duty cycle, intensity of SATA 0.5 W, 16 J/cm^2; Chattanooga Group Inc., Chattanooga, TN, USA) every other day, beginning on the day of surgery (Fig. 2). The final 32 rats received both LLLT and LIPUS therapies with consecutive treatment protocols. On day 14 post-injury, half of the animals from each group were sacrificed by means of an intraperitoneal lethal dose of anaesthetic agent in order to extract their right tibias; the remaining animals were sacrificed on day 21. After sacrifice, eight specimens from each subgroup were submitted to histological analysis and the other eight were submitted to a three-point bending test.

Histological analysis

Tibias were dissected and fixed in 10% formaldehyde, decalcified in 10% ethylenediaminetetraacetic acid (EDTA), dehydrated in ethyl alcohol, cleared in xylene, and embedded in paraffin blocks. The specimens were then sectioned transversely using a rotary microtome (Leitz Wetzlar). Sections of 5–7 μm were obtained from the osteotomy area. These sections were stained with haematoxylin and eosin, and at least six sections were examined for each specimen. The parametric indices for bone tissue were as follows: 'new bone formation' (area of the newly formed bone as a percentage of the defect area), 'connective tissue formation' (area of the connective tissue formed as a percentage of the defect area), 'intensity of inflammation' (distribution of the lymphocytes in the defect area, scored 0–3), and 'vascularization' (quantity and form of blood vessels in the defect area, scored 0–3). The histological evaluation and analysis was conducted using a double-blind protocol.

Mechanical test

A universal testing machine was used for the three-point bending test (Shimadzu AG-IC series). A metal support was used to locate the tibias and a 5 N pre-load was applied in order to avoid specimen sliding. A bending force at a speed of 3 mm/min was then applied to perform the test. The software used (Trapezium Lite X) provided the load/displacement graph for each specimen. After the tests, the bones were positioned vertically and photographed under a stereomicroscope (Leica DFC320). The images were analyzed using the software AutoCAD 2007 and the cross-sectional area and the cross-sectional moment of inertia were calculated. The maximum stress and stiffness were then calculated.

Statistical analysis

Comparisons among groups were made using one-way analysis of variance (ANOVA), and the post hoc Tukey HSD (honestly significant difference) test was used to verify significant differences among the groups. Comparisons were made between the same time-points for

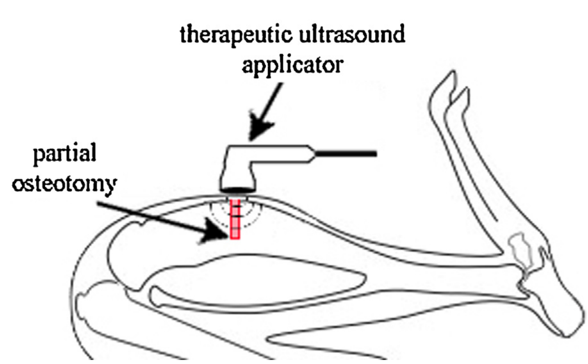

Fig. 2. Application of the therapeutic ultrasound device.